IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
GREENFIELD et al.
Serial No. 09/155076
Filed: March 21, 1997
For: "PEPTIDE FROM SOLUBLE FORM OF ACETYLCHOLINESTERASE, ACTIVE AS A CALCIUM CHANNEL MODULATOR"

DECLARATION

The Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, Martin Westwell, do hereby declare and state as follows:

1. I am a British citizen of 13 Mill Road, Abingdon, Oxfordshire OX14 5NS. I currently hold the position of Research Manager at Synaptica Limited and until 30 June 2001 I was the Research Coordinator of Synaptica Limited and held a fellowship at Lincoln College, Oxford University in biological/medicinal sciences. Synaptica Limited is the assignee of US Patent Application serial no. 09/155076.

Amended 11-26-03

2. I have read the comments of the Examiner in the Office Communication dated 22nd June 2001 concerning the above-identified US Patent Application. As a result of my position in Synaptica Limited, I have first hand-knowledge of technical data relevant to the suggestions by the Examiner that whole human acetylcholinesterase (AChE) and the 40 amino acid residue C-terminal fragment of human AChE (the product of exon 6 of the AChE gene) share the calcium channel modulatory function of Synaptica

peptide (the 14mer of SEQ. ID no. 1 as noted in US Patent Application Serial no. 09/155076). These suggestions of the Examiner are wrong as evident from technical data discussed below.

3. Synaptica peptide is contained within the C-terminal 40 mer tail sequence of human AChE (commonly referred to as the T40 peptide) but has both distinct structural and functional characteristics from that longer peptide. In particular, at nanomolar concentrations, Synaptica peptide has been shown to allosterically potentiate the response of the alpha 7 nicotinic receptor to acetylcholine and other agonists of that receptor. The T 40 peptide does not exhibit this modulatory function as shown by the data in Figure 1 below. The data in Figure 1 was obtained using the biotinylated and amidated version of Synaptica peptide. However, additional experiments documented in Published International Application WO 01/73446 of Synaptica Limited (a copy of which is annexed hereto as Exhibit 1) provide further evidence that Synaptica peptide is capable of modulating calcium flux through alpha 7 nicotinic receptors. It will be noted that I am a named inventor on that patent application.

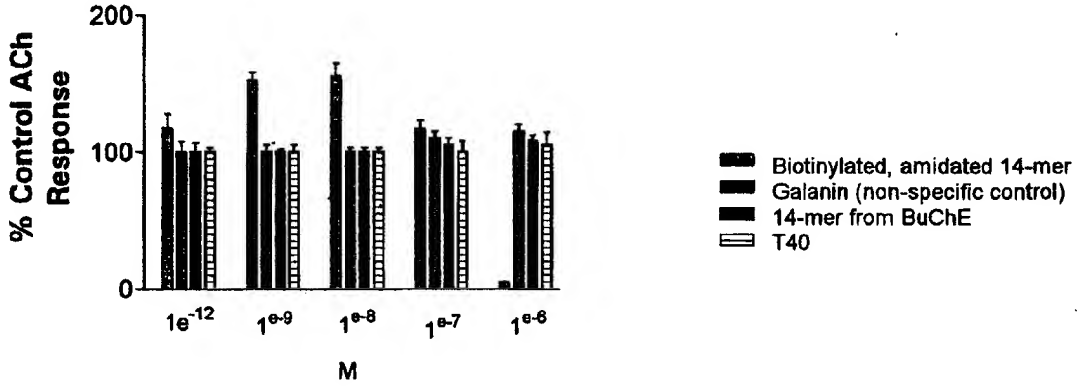


Figure 1 *The positive allosteric effect of the biotinylated and amidated 14-mer can be seen at nanomolar concentrations. The experiments were carried out using Xenopus oocytes injected with α7 nAChR RNA. Impaled oocytes were superfused with an EC50 concentration of acetylcholine (100 μM) and then 100 μM acetylcholine plus increasing concentrations of the peptides under study. Data represents the mean ± SEM of 4 independent experiments (4 different batches of oocytes)*

In keeping with the above-noted different functional activity of T40 peptide and Synaptica peptide, circular dichroism spectroscopy shows that an α-helical structure

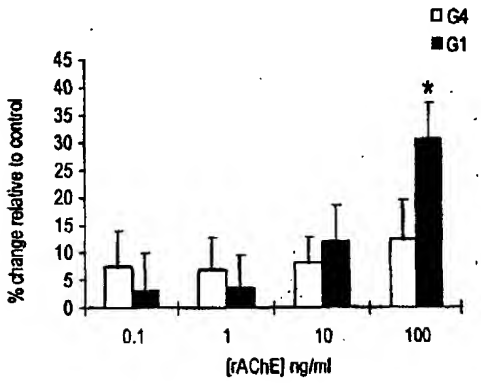
dominates the T40 peptide whereas Synaptica peptide is random coil, β-turns or β-sheet depending upon the conditions.

4. The Examiner is correct that Example 3 of US Patent Application Serial no. 09/155076 suggests that whole AChE causes calcium influx into neurons. However, further experiments such as those described above in Figure 1 indicate that this does not reflect that AChE shares the same non-enzymic mechanism of action as Synaptica peptide. The AChE protein is not a functional analogue of Synaptica peptide. This conclusion is consistent with experiments looking at the ability of G_1 AChE (the recombinant monomeric form of terameric AChE minus the T40 tail) and Synaptica peptide to influence neurite outgrowth of cultured neuronal cells. On cultured hippocampal cells, Synaptica peptide at 1 to 10 nM causes a brief period of neurite outgrowth prior to apoptosis (cell death). Increasing the concentration and/or incubation time of the 14 mer causes a clear apoptotic-necrotic continuum (see Table 1 below). This can be explained in terms of change of calcium flux into the neurons. In contrast, full length G_1 AChE (3 U/ml) causes a robust neurotrophic response (see Figure 2 below) consistent with a different non-cholinergic action from Synaptica peptide. In the same system, T40 peptide has no response on cell survival and/or health.

Incubation time (hours)	[Synaptica peptide]	Mode of cell death
1	1 – 10 nM	Compensatory
24	1 nM – 1 mM	No effect
48	100 nM – 1 μM	Apoptosis
72	10 nM – 1 μM	Apoptosis
72	10 μM – 1 mM	Necrosis
336	10 μM – 1 mM	Necrosis
336	1 nM – 1 μM	Apoptosis

Table 1 *The action of the 14-mer peptide on cultured hippocampal neurons is dependent on dose and incubation time.*

Figure 2: the non-cholinergic ability of AChE to enhance neurite outgrowth of cultured neurons. Organotypic neurons of the substantia nigra were used to show the neurotrophic effect of monomeric AChE. This effect is significant at 100ng/ml.



5. The conclusion must be that processing of whole AChE or the T40 peptide is required to produce the biological activity exhibited by Synaptica peptide. In support of such processing underlying linkage of AChE to neurodegenerative disease causation, it has been shown that Synaptica peptide can be injected into the brains of rats to cause attentional deficit reminiscent of Alzheimer's disease. Such studies are documented in Published International Application no. WO 01/49107, also in the name of Synaptica Limited, a copy of which is annexed as Exhibit 2.

6. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of this declaration, the patent application, or any patents issuing thereon.

Martin Westwell

18 October 2001
Date